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(54) Title: CYCLOOXYGENASE REGULATION WITH PG LIPOSOMES

(57) Abstract: Cyclooxygenase-related inflammatory and pain-causing disorders in a mammalian subject can be alleviated by reducing the pathological expression of the COX-2 gene in the mammalian patient, by administration thereto of bodies, such as liposomes, beads or similar particles, of a size and conformation resembling mammalian apoptotic cells or apoptotic bodies, and which carry as a major component on their surface at least one phospholipid having as exteriorly presented head groups phosphato glycerol and synthetic mimetics thereof.

WO 2004/037270 A1

Cyclooxygenase Regulation with PG Liposomes

Field of the invention

This invention relates to the enzyme cyclooxygenase, and methods for its regulation in the mammalian body. More specifically, it relates to a process and composition for administration to a mammalian subject for modulating expression of the expression of the cyclooxygenase (COX)-2 gene. Such modulation can be advantageously employed in the overall well-being of the mammalian body, especially in alleviation of pain.

Background of the invention and prior art

Cyclooxygenase (COX, also referred to as prostaglandin endoperoxide H synthase, PGHS) is the rate-limiting enzyme in prostaglandin synthesis in the body, and exists as two isoforms, COX-1 and COX-2. They have two enzymatic functions, namely a cyclooxygenase function that converts arachidonic acid to prostaglandin G_2 (PGG_2), with formation of an oxygen-containing ring structure, and a peroxidase activity that converts PGG_2 to prostaglandin H_2 (PGH_2). PGH_2 in turn acts as the precursor of various eicosanoids, including PGE_2, $PGF_{2\alpha}$, PGD_2, prostacyclin and thromboxane A_2. Eicosanoids are commonly known as pivotal regulators of immune and inflammatory processes (Gong C, et al, Brain Res. 2001 901:38-46). It is known that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) block the enzymatic activity of both isoforms. This is at least part of the underlying mechanism of the anti-inflammatory activity of aspirin and other

NSAIDs, prostaglandins being, in general, inflammation stimulating substances.

The enzymes COX-1 and COX-2 are encoded by separate genes located on different chromosomes. The COX-1 gene is larger, and its structure facilitates continuous transcription of a stable message, so that it is a constitutive, housekeeping enzyme, present in most cells and tissues. The COX-2 gene is smaller, and is consistent with its characterization as an immediate-early stage gene that is rapidly upregulated during inflammation and other pathological processes. COX-2 normally is not detectable in most tissues, but can be induced by mitogens, cytokines and certain inflammatory agents.

A consequence of the over expression or over-activity of either or both of COX-1 and COX-2 is pain. The source or cause of the pain may be inflammation, tissue damage through injury, muscle damage through injury, over-exertion, etc., burns, chemical exposure, toxic substance ingestion or the like, all of which are associated with increases in COX-2 activity at the site of origin of the pain.

Treatment of inflammation in a patient by administration of NSAIDs is well known. These inhibit both isoforms due to their occupancy of the COX active site, preventing access by arachidonic acid. The resulting local inhibition of prostaglandins, however, is believed to be the underlying cause of unwanted

gastro-intestinal side effects such as stomach bleeding and ulceration. This is attributed to inhibition of COX-1, whereas the isoform COX-2 is the one closely associated with inflammation since it mediates production of prostaglandin. Various selective inhibitors of COX-2 have accordingly been developed, such as celecoxib and rofecoxib, to treat inflammation, with reduced gastro-intestinal side effects. The COX-2 selective inhibitors, however, have been reported to cause different side effects, most notably in the cardiac area. One concern is the reported increased risk of myocardial infarction and thrombosis in patients on selective COX-2 inhibitors. This increase may be a consequence of the reduction of the formation of prostaglandin PGI_2 as a result of selective COX-2 inhibition, whilst leaving the thromboxane A2 producing activity of COX-1 unaffected. PGI_2 functions as an inhibitor of platelet aggregation, whilst thromboxane A2 functions as a promoter of platelet aggregation. Celecoxib, in particular, has been shown to lower prostacyclin without affecting thromboxane. This tips the balance of prostacyclin/thromboxane in favour of thromboxane leading to increased vascular and thrombotic events (Bing RJ et al, J Am Coll Cardiol 2002 39:521-2). A large-scale study with the COX-2-specific inhibitor rofecoxib (Vioxx Gastrointestinal Outcomes Research [VIGOR] trial) revealed that rheumatoid arthritis patients taking rofecoxib had a greater incidence of cardiovascular events than patients taking the NSAID naproxen (DeMaria AN, Am J Cardiol 2002 89:33-8).

Certain pathophysiological conditions, including ischemia and hypoxia, are associated with increased expression of COX-2 in the brain. It has been

shown that topical administration of lipopolysaccharide (LPS) caused a time-dependant dilatation of cerebral arterioles that was inhibited by the selective COX-2 inhibitor NS-398 (Brian JE Jr. et al, Stroke 1998 29:2600-6). Vasodilatation is one of the cardinal manifestations of inflammation in the brain. In an experimental model of brain inflammation COX-2 was shown to be expressed in endothelial cells and it has been suggested that it plays a role in the development of fever during brain inflammation (Cao C et al, J Neurosci 1999 19:716-25).

COX-2 has also been suggested to be the key element controlling the generation of proinflammatory mediators involved in the progression of Alzheimer's disease (AD). In mouse studies, rofecoxib was shown to significantly reverse LPS-induced retention deficit. LPS is known to induce the in vivo synthesis and release of inflammatory mediators such as IL-1, IL-6 and PGE_2 (Jain NK et al, 2002 Behavioural Brain Research 133:369-376). Inhibitors of COX-2 have also been shown to be effective as therapeutic agents in experimental autoimmune neuritis.

A procedure or composition which, when administered to mammalian patients, modulates the expression of the COX-2 gene should, therefore, provide a basis for a treatment or inhibition of the progression of a variety of inflammatory and pain-causing disorders in mammalian patients and of the pain associated therewith.

It is an object of the present invention to provide a composition and a method for treating a mammalian subject, to modulate pathological COX-2 activity, and thereby alleviate COX-2-related disorders, with reduced adverse side effects.

Summary of the invention

It has now been found that a cyclooxygenase-related pain-causing disorder in a mammalian subject can be alleviated by reducing the pathological expression of the COX-2 gene in the mammalian patient, by administration thereto of bodies, such as liposomes, beads or similar particles, of a size and conformation resembling mammalian apoptotic cells or apoptotic bodies, and which carry as a major component on their surface at least one phospholipid having as exteriorly presented head groups phosphato glycerol and synthetic mimetics thereof. The lipid portion of the phospholipid can be chosen from a wide rage of fatty acids, saturated and unsaturated, for example dioleyl, distearoly, dipalmitoyl, oleyl-stearoyl, oleyl-palmitoly, stearoyl-palmitoyl, etc, of approproiate carbon chain length, as commonly found in phospholipids. They can be synthetic or natural products. Preferred examples are phospholipids selected from phosphatidylglycerol, distearoylphosphatidylglycerol and dipalmitoylphosphatitidylglycerol. The bodies may carry, as a minor component on their surface, an inactive constituent.

Thus according to a first aspect of the present invention, there is provided a composition of matter useful in inhibiting COX-2 expression in a mammalian patient following administration thereto, the composition comprising:

pharmaceutically acceptable bodies having a three-dimensional core, of conformation and size corresponding to mammalian apoptotic cells and/or

apoptotic bodies, said bodies having a COX-2 expression inhibiting amount of a ligand comprising a phospholipid having a phosphato glycerol head group expressed or expressible on the surface thereof.

According to a second aspect of the invention, there is provided a process for treating a mammalian patient to alleviate the symptoms of a COX-2 related disorder, which comprises administering to the patient a COX-2 expression inhibiting amount of pharmaceutically acceptable bodies having a three-dimensional core, of conformation and size corresponding to mammalian apoptotic cells and/or apoptotic bodies, said bodies having a COX-2 expression inhibiting amount of a ligand comprising a phospholipid having a phosphato glycerol head group expressed or expressible on the surface thereof.

A further aspect of the invention provides use, in the manufacture of a medicament for administration to mammalian patients for alleviating the symptoms of a COX-2 related disorder, of pharmaceutically acceptable bodies having a three-dimensional core, of conformation and size corresponding to mammalian apoptotic cells and/or apoptotic bodies, said bodies having a COX-2 expression inhibiting amount of a ligand comprising a phospholipid having a phosphato glycerol head group expressed or expressible on the surface thereof.

Brief description of the drawings

Figure 1 shows the mRNA levels of cyclooxygenase (COX)-2 measured by a semi-quantitative polymerase chain reaction (PCR) method in a macrophage established cell line system, Example 1 below.

Detailed Description of the Invention

Preferred phospholipids for use in the present invention are selected from phosphatidylglycerol, distearoylphosphatidyglycerol and dipalmitoylphosphatidyglycerol.

In a preferred embodiment, the invention from one aspect is directed to a composition comprising bodies having a three-dimensional core structure of conformation and size corresponding to mammalian apoptotic cells and /or bodies and expressing or expressible on the surface thereof phosphato glycerol head group-containing ligands which are chosen so as to interact with certain receptors on the cells of the mammalian body, on administration to a mammalian patient, with the result that the COX-2 expression in the patient is modulated. "Modulating" is defined to mean reducing, physiologically restoring, normalizing or controlling.

As a result of their interacting with certain receptors on the cells of the mammalian body, it is believed that the compositions of the present invention initiate a series of events in the immune system of the mammalian body. While it is not intended that the present invention should be limited by any

particular theory of its mechanism or mode of action, it is believed that the bodies of the composition are phagocytosed by antigen presenting cells (APCs) of the immune system with consequent alteration of the cytokine profile of the APCs in favour of COX-2 expression reduction.

In addition, the COX-2 modulation effected by the present invention may be the result of analgesic mechanisms in the patient's body. Accordingly, the compositions of the invention are indicated for us in analgesia, pain management and anti-pruritic therapy.

"Analgesia" is defined as alleviation of pain, acute and chronic, induced by all known mechanisms, including but not limited to pain related to postoperative pain, cancer, neuropathic pain, and complex regional pain syndrome.

Another preferred embodiment is a method for treating non-inflammatory related pain involving excessive COX-2 expression in a mammalian patient comprising administering to a mammalian patient a non-toxic, effective, excessive-COX-2-expression- treating amount of liposomal or non-liposomal bodies as defined above, wherein the pain is inhibited and/or reduced.

Examples of three-dimensional body portions include liposomes, solid beads, hollow beads, filled beads, particles, granules, microspheres, or beads

of biocompatible materials, natural or synthetic, such as polyethylene glycol, polyvinylpyrrolidone, polystyrene, poly(methylmethacrylate), etc., polysaccharides such as hydroxyethyl starch, hydroxyethylcellulose, agarose and the like, as commonly used in the pharmaceutical industry. The beads may be solid or hollow, or filled with biocompatible material. These synthetic and semi-synthetic bodies are three dimensional bodies having shapes and dimensions ranging from those resembling mammalian cells to shapes and dimensions approximating to apoptotic bodies produced by the apoptosis of mammalian cells (typically but not exclusively spheroidal, cylindrical, ellipsoidal, including oblate and prolate spheroidal, serpentine, reniform etc., and having diameters of about 20 nanometres to about 500 microns), and having COX-2 expression reducing phosphato-glycerol containing ligands on the surface thereof.

Preferred compositions of matter are liposomes, which may be composed of a variety of lipids. Liposomes, or lipid vesicles, are sealed sacs, in the micron or sub-micron range, the walls of which consist of suitable amphiphiles. They normally contain an aqueous medium. Liposomes are vesicles which can be unilamellar (single membrane) or multi-lamellar (onion-like structures characterized by multiple membrane bilayers, each separated from the next by an aqueous layer). The bilayer is composed of two lipid monolayers having a hydrophobic "tail" region and a hydrophilic "head" region. In the membrane bilayer, the hydrophobic (nonpolar) "tails" of the lipid monolayers orient toward the center of the bilayer, whereas the hydrophilic

(polar) "heads" orient toward the aqueous phase. Generally the liposomes are composed of phosphatidylglycerol, distearoylphosphatidylglycerol, and dipalmitoylphosphatidylglycerol. Such liposomes are prepared and treated so that the active polar head groups are presented exteriorly on the liposomal body. .

Methods of preparing liposomes of the appropriate size are known in the art and do not form part of this invention. Reference may be made to various textbooks and literature articles on the subject, for example, the review article "Liposomes as Pharmaceutical Dosage Forms", by Yechezkel Barenholz and Daan J. A. Chrommelin, and literature cited therein, for example New, R. C. "Liposomes: A Practical Approach", IRL Press at Oxford University Press (1990).

The diameter of the ligand-carrying liposomes of the preferred embodiment of this invention is from about 20 nanometers to about 500 microns, more preferably from about 20 nanometers to about 1000 nanometers, more preferably from about 50 nanometers to about 500 nanometers, and most preferably from about 80 nanometers to about 120 nanometers. Such preferred diameters will generally correspond to the diameters of mammalian apoptotic bodies or apoptotic cells (typically from 20 nanometres to 500 microns in diameter as measured along the longest axis).

When biocompatible non-liposomal synthetic bodies are chosen for use,

these may be selected from polysaccharides such as hydroxyethylcellulose, hydroxyethyl starch, agarose; or from non-polysaccharide materials such as polyethylene glycol, poly(methylmethacrylate), polyvinylpyrrolidone, polystyrene and a wide range of other natural, semi-synthetic and synthetic materials.

Suitable substances for derivatization to attach the phospholipid(s) to three-dimensional bodies are commercially available e.g. from Polysciences Inc., 400 Valley Road, Warrington, PA 18976, or from Sigma Aldrich Fine Chemicals.

In another embodiment, this invention is directed to lyophilized or freeze-dried bodies carrying such anti-inflammatory promoting ligands, and kits comprising lyophilized or freeze dried bodies carrying such anti-inflammatory promoting ligands and a pharmaceutically acceptable carrier.

The three-dimensional bodies as defined above have one or more ligands selected from phosphato glycerol head groups, preferably selected from phosphatidylglycerol, distearoylphosphatidylglycerol and dipalmitoylphosphatidylglycerol on the exterior surface in a manner, in one embodiment, that they are believed to be capable of interacting with the appropriate receptor(s), on antigen presenting cells *in vivo,* with resulting modulation of expression of COX-2 in the mammalian patient, following administration thereto. The structure of these ligands may be synthetically altered and include all, part of or a modified version of the original ligand.

Thus a preferred embodiment of this invention provides synthetic or semi-synthetic bodies carrying anionic and other phospholipids ligands on their surface, namely liposomes or non-liposomal bodies which expose or can be treated or induced to expose on their surface phosphato glycerol head groups, preferably phosphatidylglycerol, distearoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, and the use thereof in treating COX-2 and pain-inducing disorders in mammalian patients. The structure of these ligands may be synthetically altered and include all, part of or a modified version of the original ligand.

The preferred phospholipid for use in the present invention is phosphatidylglycerol optionally with a minor portion (up to less than 50% by weight) of another, inactive constituent. The preferred liposome is one constituted to the extent of about 50-100% by weight of phosphatidylglycerol (PG), the balance being phosphatidylcholine (PC) or other such biologically acceptable phospholipid(s). More preferred are liposomes constituted to the extent of 65-90% by weight, most preferably 70-80% by weight, with the single most preferred embodiment being about 75% by weight of active phospholipids, the balance being the other inactive phospholipids such as PC. It is also within the scope of the present invention to use bodies having a mixture of the aforementioned phospholipids having chemically active head groups, this mixture comprising at least 10%, preferably at least 50% and most

preferably 60-90% of the aforementioned active phospholipids.

Analogues of phosphatidylglycerol with modified active head groups, which also interact with the PG receptors on the antigen presenting cells, or otherwise result in a COX-2 expression modulation reaction in the recipient body, are contemplated within the scope of the term phosphatidylglycerol.

It is contemplated that the patient may be a mammal, including but not limited to humans and domestic animals such as cows, horses, pigs, dogs, cats and the like.

Phospholipids are amphiphilic molecules (i.e. amphiphiles), meaning that the compound comprises molecules having a polar water-soluble group attached to a water-insoluble hydrocarbon chain. The amphiphiles serving as the layers of the matrix have defined polar and apolar regions. The amphiphiles can include in addition to the phospholipids providing the active head groups in the process of the invention, other, naturally occurring lipids used alone with the phospholipid carrying the active head group, or in a mixture with another. The amphiphiles serving as the layer(s) of the liposomes can be inert, structure-conferring synthetic compounds such as polyoxyethylene alkylethers, polyoxyethylene alkylesters and saccharosediesters.

The ligand-carrying bodies may be suspended in a pharmaceutically

acceptable carrier, such as physiological sterile saline, sterile water, pyrogen-free water, isotonic saline, and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations. Preferably, the ligand-carrying bodies are constituted into a liquid suspension in a biocompatible liquid such as buffered saline and administered to the patient in any appropriate route which introduces it to the immune system, such as intra-arterially, intravenously, intra-dermally, or most preferably intramuscularly or subcutaneously.

It is contemplated that the ligand-carrying bodies may be freeze-dried or lyophilized so that they may be later resuspended for administration. A protectant such as sucrose or trehalose is preferably included in any such freeze-dried composition, to protect the liposomes from damage on re-suspension. This invention is also directed to a kit of parts comprising lyophilized or freeze-dried ligand-carrying bodies and a pharmaceutically acceptable carrier, such as physiological sterile saline, sterile water, pyrogen-free water, isotonic saline, and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations.

A preferred manner of administering the ligand-carrying bodies to the patient is a course of injections, administered daily, several times per week, weekly or monthly to the patient, over a period ranging from a week to several months. The frequency and duration of the course of the administration is likely to vary from patient to patient, and according to the condition being treated, its

severity, and whether the treatment is intended as prophylactic, therapeutic or curative. Its design and optimization is well within the skill of the attending physician. Intramuscular injection is most preferred. One particular injection schedule, in at least some of the indications of the invention, is an injection of an appropriate amount of bodies on day 1, a further injection on day 2, a further injection on day 14, and then "booster" injections at monthly intervals.

The quantities of ligand-carrying bodies to be administered will vary depending on the nature of the mammalian disorder it is intended to treat and on the identity and characteristics of the patient. It is important that the effective amount of ligand-carrying bodies is non-toxic to the patient, and is not so large as to overwhelm the immune system. When using intra-arterial, intravenous, subcutaneous or intramuscular administration of a liquid suspension of ligand-carrying bodies, it is preferred to administer, for each dose, from about 0.1-50 ml of liquid, containing an amount of ligand-carrying bodies generally equivalent to 10% - 1000%, preferably 10% - 100%, of the number of leukocytes normally found in an equivalent volume of whole blood or the number of apoptotic bodies that can be generated from them. Generally, the number of ligand-carrying bodies administered per delivery to a human patient is in the range from about 500 to about 2.5×10^{12} (<250 ng of bodies, in the case of liposomes, pro-rated for density differences for other embodiments of bodies), more preferably from about 1,000 to about 1,500,000,000, even more preferably 10,000 to about 100,000,000, and most preferably from about 200,000 to about 20,000,000.

The invention is indicated for use with a wide range of disorders associated with an increase in COX-2 activity including, but not limited to pancreatitis, endometriosis, and adenomyosis.

The invention is indicated in the inducement of analgesia and the treatment of pain, especially pain unrelated to or not substantially related to inflammation. The source or cause of the pain may include but is not limited to, tissue damage through injury, muscle damage through injury, over-exertion, etc., burns, chemical exposure, toxic substance ingestion or the like, all of which are associated with increases in COX-2 expression at the site of origin of the pain.

The invention is indicated for the treatment and prophylaxis of the symptoms of a wide variety of mammalian neuroinfectious diseases including but not limited to meningitis and encephalitis.

In an alternative embodiment of the invention, the compounds of this invention may be administered prior to, in conjunction with or subsequent to administration of a pharmaceutically effective amount of a known COX-2 inhibitor, such as celecoxib (Celebrex ®) or refecoxib (Vioxx®). When said alternative embodiment is employed, the pharmaceutically effective amount of the COX-2 inhibitor is administered to reduce excessive COX-2 activity. A pharmaceutically effective amount is well known in the art.

Thus the present invention is applicable for treatment of substantially any excessive COX-2 expression-related non-inflammatory pain condition. COX-2 is constitutively expressed in neurons. Excessive COX-2 expression may result from cell damage such as physical injury, chemical injury and pressure on body organs resulting from tumour growth or organ enlargement due to malignancies or other disorder. Such pain is experienced substantially instantaneously, as a result of excessive COX-2 at the site, the consequent release of pain "Substance P", from the peripheral sensory nerve endings, to the nerves and the spinal cord and thence to the brain. In contrast, pain due to inflammation is slow to build as the inflammation develops and becomes chronic rather than acute.

Another example of a disorder well-suited to treatment according to the invention is post-herpetic neuralgia resulting from shingles where extreme pain develops as an after-effect. Persons developing hyperalgesia (extreme sensitivity to pain) are also suitably treated by the invention.

Pressure due to organ enlargement is effectively a mechanical pressure, the pain from which is non-inflammatory and intractable. Organ linings, e.g. of the liver, contain cells with substance P, which is released in association with excessive expression of COX-2 therefrom. Similarly, bone pressures associated with cancer growths create pain by similar mechanisms. These are treatable according to the invention.

See Vanagash et al., Progr. in Neurobiology, 2001 July: 64: 327-63, contents incorporated herein.

See also Camu F et al., Drugs. 2003; 63 Suppl 1: 1-7; Ruoff et al., J. Pain Symptom Manage. 2003 Feb; 25 (2 Suppl): S21-31; Kiefer et al., Cum. Opin Investig Drugs 2002 Sep; 3(9): 1348-58; and Sinatra R, J. Pain Symptom Manage. 2002 July; (1 Suppl): 518-27, all of which are incorporated herein by reference.

The invention is further described, for illustrative purposes, in the following specific example.

Example

U937 is a monocytic leukemia line that can be differentiated into macrophages by administration of a phorbol ester. Treatment with lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, stimulates a response in U937 cells, with the upregulation of expression of a number of molecules including COX2. This provides an experimental system for the assessment of COX-2 related therapies. The macrophages can be grown in culture medium in the presence of a suspected COX-2 affecting composition, and the expression of RNA or DNA of the COX-2 measured.

Liposomes of size 100 ± 20 nanometers were prepared according to standard

methods known in the art and had a composition of 75% phosphatidylglycerol (PG), 25% phosphatidylcholine (PC). The stock concentration of liposome was 39.5mM lipid and was diluted to the following final concentrations in the assay:

100μM phosphatidylglycerol (PG)

39.8μM PG

10μM PG

3.98μM PG

1μM PG

The U937 cells were cultured by growing in RPMI medium (GIBCO BRL) with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin at 37°C, in an atmosphere containing 5% CO_2. They were seeded into 6 well plates at a concentration of 5×10^5 cells per ml with 2 mls of cells added per well and differentiated into macrophages by treating with 150nM phorbol myristate acetate (PMA) for 2-3 days. The cell medium was then replaced with complete medium after the U937 cells had differentiated into macrophages. The cells were incubated for a further for 24hrs prior to liposome addition, so as to allow any up-regulation of genes/proteins induced by PMA to be reduced.

The cells were then incubated with either:

Phosphate buffered saline (PBS) – as a negative control,

10ng/ml Lipopolysaccharide (LPS) – as a positive control,

10ng/ml LPS + 100μM PG,

10ng/ml LPS + 39.8μM PG,

10ng/ml LPS + 10μM PG,

10ng/ml LPS + 3.98μM PG,

or 10ng/ml LPS + 1μM PG.

The cells were incubated at 37°C, 5% CO_2. After 18hrs, each culture was harvested; the cells were rinsed with PBS and lysed with 1ml of Tri-Reagent ™ (Sigma) for mRNA analysis. COX-2 and GAPDH mRNA were extracted and detected with the reverse transcription-polymerase chain reaction as (RT-PCR) and carried out using standard methods known in the art (Adderley SR, Fitzgerald DJ, J Biol Chem, Vol. 274, Issue 8, 5038-5046, February 19, 1999). The reaction was performed using the following primers:

Human 847bp COX-2 Primers

Sense: **5'** TCC TTG CTG TTC CCA CCC ATG **3'**

Antisense: **5'** CAT CAT CAG ACC AGG CAC CAG **3'**

Human GADPH Primers

Sense: **5'** CCA CCC ATG GCA AAT TCC ATG GCA **3'**

Antisense: **5'** TCT AGA CGG CAG GTC AGG TCC ACC **3'**

The GADPH was included as an experimental control to allow for the rough quantitation of COX-2 in the assay.

Results

Lane 1 on gel contains markers that confirm the size of the COX-2/GADPH bands. There is a constant band in all lanes of the GADPH gel as this is a housekeeping gene and provides a control for the loading of the gel. The control lane indicates those cells that were not treated with LPS and shows a very low level of COX-2 expression. LPS treatment causes a measurable increase in COX-2 mRNA production (lane 3) when compared with control. This LPS-induced expression is significantly reduced by 100μM and 38.9μM 75% PG and is reduced to a lesser extent by 10μM - 1μM 75% PG.

What is claimed is

1. Use in preparation of a medicament for treating pain of a predominantly non-inflammatory related nature and consequent upon excessive COX-2 expression, in a mammalian patient, of composition of matter comprising:

pharmaceutically acceptable bodies having a three-dimensional core structure of conformation and size corresponding to mammalian apoptotic cells and/or bodies, and expressing or expressible on the surface thereof, a COX-2 expression inhibiting amount of a ligand comprising a phospholipid having a phosphato glycerol head group expressed or expressible on the surface thereof.

2. Use according to claim 1 wherein the phospholipid is selected form phosphatidylglycerol, distearoylphosphatidylglycerol and dipalmitoylphosphatidylglycerol, and mixtures thereof.

3. Use according to claim 2 wherein the phospholipid is phosphatidylglycerol.

4. Use according to claim 1, claim 2 or claim 3, wherein said pharmaceutically acceptable body is selected from the group consisting of hollow beads, filled beads, solid beads, particles, microspheres, granules and liposomes.

5. Use according to claims 1-4, wherein the pharmaceutically acceptable synthetic or semi-synthetic body is a liposome.

6. Use according to any preceding claim, wherein said bodies range in size from about 20 nanometers to about 500 microns.

7. Use according to any preceding claim, of a unit dosage of composition comprising from about 10,000 to 2,500,000,000 of said bodies.

8. Use according to any preceding claim, wherein said composition further comprises a pharmaceutically effective amount of celecoxib or rofecoxib.

9. Use according to any preceding claim for inducing analgesia in treatment of a cyclooxygenase-2 related disorder.

10. Use according to any of claims 1-8, in treatment of a predominantly non-inflammatory pain causing condition in a mammalian patient.

11. Use according to claim 10 wherein the condition is pancreatitis.

12. Use according to claim 10 wherein the condition is endometriosis.

13. Use according to claim 10 wherein the condition is adenomyosis.

14. Use according to claim 10 wherein the condition is tissue damage from injury, muscle damage from injury, over-exertion, burn, chemical exposure, or toxic substance ingestion.

15. Use according to claim 10 wherein the condition is meningitis.

16. Use according to claim 10 wherein the condition is encephalitis.

17. Use according to claim 10 wherein the condition is post-herpetic.

18. Use according to claim 10 wherein the condition is hyperalgesia.

19. Use according to claim 10 wherein the condition is organ enlargement
pressures or tumour growths due to cancer.

20. A method of treating pain of a predominantly non-inflammatory related nature and consequent upon excessive COX-2 expression which comprises administering to a mammalian patient suffering or at risk of suffering therefrom an effective amount of a composition of matter comprising:
pharmaceutically acceptable bodies having a three-dimensional core structure of conformation and size corresponding to mammalian apoptotic cells and/or bodies, and expressing or expressible on the

surface thereof, a COX-2 expression inhibiting amount of a ligand comprising a phospholipid having a phosphato glycerol head group expressed or expressible on the surface thereof.

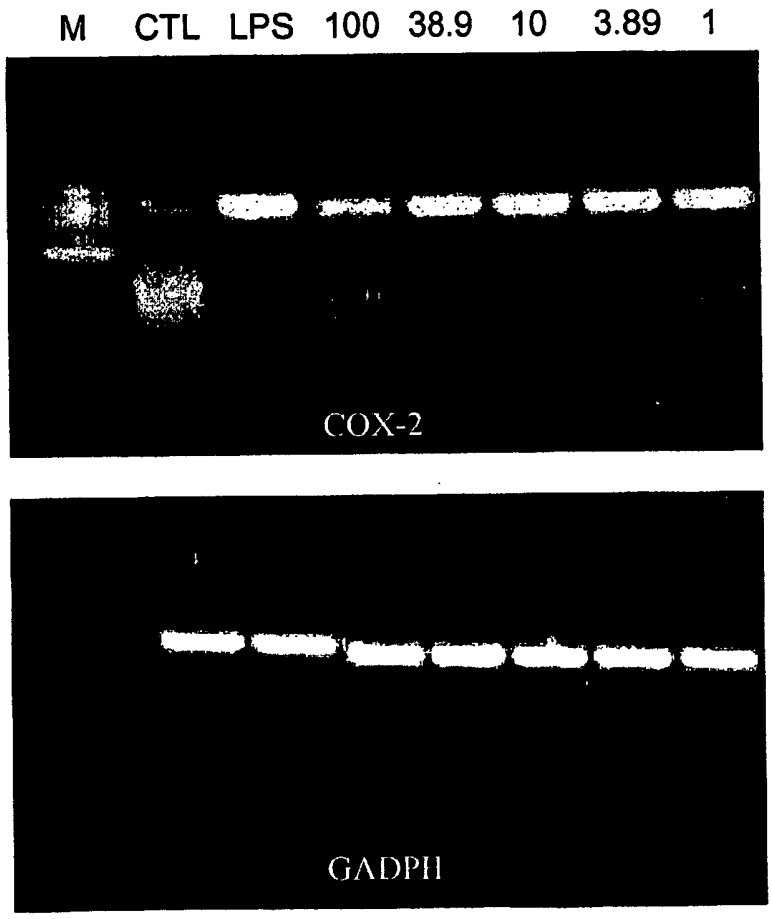
21. A method for inducing analgesia to treat a cyclooxygenase-2 related disorder comprising administering to a patient a clinically acceptable effective cyclooxygenase disorder-treating amount of a pharmaceutical composition as used in any of claims 1-8.

22. The method of claim 21 wherein the amount of said pharmaceutically acceptable bodies is less than 30 mg per kg patient body weight.

23. The method of 21 wherein the number of said bodies administered is from about 500 to about 2.5×10^{12}.

Figure 1

Dose Response to PG



INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01622

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/685 A61K31/56 A61K31/42 A61K9/127 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 90 11781 A (ALCON LAB INC) 18 October 1990 (1990-10-18) the whole document	1-6,9, 10,14, 20,21 7,8, 11-13, 15-19, 22,23
X Y	WO 01 03668 A (SHEK PANG N ;ZAMECNIK JIRI (CA); HUNG ORLANDO (CA); TIKUISIS PETER) 18 January 2001 (2001-01-18) page 5, line 15-22 page 6, line 27 claims 1,2,9,10	1-6,9, 10,14, 20,21 7,8, 11-13, 15-19, 22,23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 February 2004	23/02/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Greif, G

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01622

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 03 086351 A (ESPERION LUV DEV INC) 23 October 2003 (2003-10-23) paragraph '0022! - paragraph '0029! paragraph '0035! paragraph '0036! page 46 paragraph '0058! paragraph '0060! claims 1,12,14	1-6, 9-11,14, 20,21
P,X	WO 03 015698 A (CHUANG YAO-CHI;HUANG LEAF ; UNIV PITTSBURGH (US); YOSHIMURA NAOKI) 27 February 2003 (2003-02-27) page 14, line 4 -page 17, line 5 page 18, line 15-22 claims 1,11,14,25,26,35,37,43,60,66,70-72	1-6,9, 10,20,21
Y	WO 02 05848 A (BANDYOPADHYAY REBANTA ;EVELETH DAVID (US); HAARLEM TOM VAN (US); P) 24 January 2002 (2002-01-24) page 13, line 2 -page 15, line 17 page 69, line 4 claims 1,2,4,6	1-23
Y	CANNON G W: "Rofecoxib: a specific cyclooxygenase inhibitor." DRUGS OF TODAY (BARCELONA, SPAIN: 1998) SPAIN APR 2000, vol. 36, no. 4, April 2000 (2000-04), pages 255-262, XP009025825 ISSN: 0025-7656 the whole document	1-23

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